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The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

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Associate Editors : Kakuji GOTŌ and Yoshihiko MATSUYAMA.

ON THE RELATIVE NUTRITIVE VALUE OF VARIOUS CARBOHYDRATES AND RELATED COMPOUNDS.

By

HISASI ARIYAMA and KATUO TAKAHASI

(From the Agricultural Chemical Laboratory of Tokio Imperial University, Komaba.)

(Received Dec. 11 th., 1929).

The authors determined the relative nutritive value of different carbohydrates and related compounds by feeding experiments with albino rats by some different method from that of Ozaki. (Nippon Nogeikwagaku-kwaishi, **2**, 845, 1926). For this purpose the following 29 compounds were used :

Starch, Agar-Agar, Inulin, Dextrin, Glycogen, Funkan, Konjak powder, Saccharose, Maltose, Lactose, d–Glucose, d–Fructose, d–Galactose, d–Mannose, d–Glucosamine, α–Methylglucoside, l–Xylose, l–Arabinose, Dioxyacetone, d–Mannite, Glycerin, Ethyleneglycol, d–Gluconic acid, d–Saccharic acid, Mucic acid, Glyceric acid, Ethylalcohol, d, l–Alanine, l–Phenylalanine,

The basal ration used in this experiment consisted of:

Meat protein	27%
Butter	70%
McCollum salt mixture	3%

(As the source of vitamin B, 0.2 g. of dry yeast was given per day per rat.)

Thus the diet was very poor on carbohydrate, neverthless the albino rats fed on this diet ad. libitum attained nearly normal growth, as shown in Chart I (A). But when it is reduced to 4.2 g. daily the animals grew up to 80∼90 g. but no further. (Chart I (B))

Experiment I.

The carbohydrates tested were :

Starch, Agar–Agar, Inulin, Dextrin, Saccharose, Maltose, Lactose.

Feeding period : April to July 1928.

Average room temperature : 23C.

Young male albino rats of 40 to 45 g. were kept in a wire cage separately and were previously fed with 4.2 g. of the basal ration for about one week. Then each 1.25 g. of the samples to be tested were supplimented to it.

The rate of growth of animals during 70 days was as follows : (Chart II)

Samples	Average gain of the body weight.	
	for 70 days (g.)	per day (g.)
Maltose	114.0	1.63
Starch	95.7	1.37

Dextrin	89.3	1.28
Lactose	89.0	1.27
Saccharose	81.3	1.26
Inulin	79.8	1.14
Agar-Agar	60.0	0.86
Basal ration	62.5	0.89

As the above result shows, the relative value is in the following order: Maltose, Starch, Dextrin, Lactose, Saccharose, Inulin and Agar-Agar; Maltose being the best. The addition of Agar-Agar had almost no effect upon the growth.

Experiment II.

In the second experimint the following substances were tested:

Glucose, Fructose, Galactose, Mannose, α–Methylglucoside, Xylose, Arabinose, Mannite, Glycerin, Ethyleneglycol. (For the control group, Starch and Dextrin were simultaneously tested.)

Feeding period: May to August 1928.
Average room temperature: 27°C.

In this experiment the animals were fed with 4.2 g. of the basal ration, as long as the animals continue to grow and when the growth was stopped 1.25 g. of each sample were added to the basal ration, then the animals began to grow up again.

Thus, the gain of the body weight after the addition of the samples may be taken as the measure of their nutritive value. The average body weight attained with 4.2 g. of the basel ration was 95 g. and the period needed for it was 41 days.

The average gain of the body weight for 40 days after adding the samples was as follows: (Chart III.)

Samples	Average gain of the body weight.	
	for 40 days (g.)	per day (g.)
Fructose	55.0	1.38
Glucose	52.5	1.31
Starch	52.0	1.30
Dextrin	51.3	1.28
Glycerin	49.0	1.23
Arabinose	35.7	0.89
Xylose	35.2	0.88
Galactose	34.2	0.86
Mannose	32.8	0.82
Mannite	25.8	0.65
α–Methylglucoside	19.5	0.49

As is shown in the above table, the relative value of the samples tested is in the following order: Fructose, Glucose, Starch, Dextrin, Glycerin, Arabinose, Xylose, Galactose, Mannose, Mannite and α–Methylglucoside, the last named being the lowest. Ethyleneglycol was found to be noxious. All the animals fed with 1.25 g. of this substance died on the next day. Contrary to the statements of some observers, Arabinose and Xylose are obviously nutritive and neither diuretic-nor diarrhetic action were observed when the amount of 1.25 g. of them were administered daily to rats. Mannose is also said to be diarrhetic, but such cases were not observed.

Experiment III.

The substances tested were as follows:

Gluconic acid, Saccharic acid, Mucic acid, Glyceric acid, Glycogen, Funkan, Konjak, Glucosamine, Dioxyacetone, Alanine, Phenylalanine, Ethylalcohol. For the control group, Starch and Glucose were simultaneously tested. (Gluconic–, Saccharic–and Glyceric acid were given as Ca–salts, Mucic acid as Na, K–salt and Glucosamine as HCl–salt.)

Feeding period: January to April 1929.
Average room temperature: 16.5°C.

The experiment was carried out exactly in the same way as in the second experiment. The maximum body weight of the animals fed on 4.2 g. of the basal ration was in average 80 g.

The average gain of the body weight for 40 days after adding the samples was as follows: (Chart IV.)

Samples	Average gain of the body weight.	
	for 40 days (g)	per day (g)
Ethylalcohol	49.0	1.23
Funkan	38.7	0.97
Glucose	35.3	0.88
Starch	34.3	0.86
Glycogen	32.7	0.82
Alanine	21.8	0.55
Konjak	19.5	0.49
Gluconic acid	19.5	0.49
Saccharic acid	19.3	0.48
Dioxyacetone	7.2	0.18

As is shown in the above table, Ethylalcohol secured the best growth, followed by Funkan and Glycogen as well as Glucose and Starch. Alanine, Konjak, Gluconic acid and Saccharic acid are far inferior to Glucose, Dioxyacetone being least effective. Mucic acid, Glyceric acid, Glucosamine and Phenylalanine are found to be noxious. The animals fed with 1.25 g. of them have all died after about 2 to 25 days.

When the rats were fed with 1.25 g. of absolute Alcohol mixed with basal ration, they ate it up within 30 minutes and showed no signs of drunkness moreover, the growth was far better than on Glucose and Starch.

From these results, the albino rat seems to have relatively high resistance toward Ethylalcohol and it can assimilate fair amount of alcohol.

Assimilability of the carbohydrates tested:

At the end of the feeding period the urine of the animals excreted during 18 hours after the ingestion of the carbohydrates was collected and it was tested qualitatively for reducing substances with Benedict's reagent.

The result of the test was as follows :

Carbohydrates	Reducing power of the urine	Carbohydrates	Reducing power of the urine
Starch	−	Glucose	−
Dextrin	−	Fructose	−
Inulin	−	Galactose	⧻
Agar-Agar	−	Mannose	⧺
Glycogen	−	α–Methylglucoside	+
Konjak	−	Glucosamine	⧺
Saccharose	−	Arabinose	⧻
Maltose	−	Xylose	⧻
Lactose	⧺	Dioxyacetone	⧺

The urine of the animals fed with Lactose, Galactose, Mannose, *α*–Methylglucoside, Glucosamine, Arabinose, Xylose and Dioxyacetone reduced the reagent. Thus the above mentioned carbohydrates were excreted partially in the urine without being utilized by the animals and the low assimilability of these carbohydrates may be regarded as a caure of their relatively poor effect upon the nutritive value. In spite of its low assimilability, Lactose was found much effective on the growth of the rats especially in the early part of the feeding period.

Summary.

(1) The basal ration used in the feeding experiments consisted of 27 % Meat protein, 70% Butter and 3% McCollum salt mixture. (As the source of vitamin B, 0.2 g. of dry yeast was given per day per rat.) Male albino rats were fed with 4.2 g. of the basal ration and 1.25 g. of different kinds of carbohydrates and other substances to be tested. Observing the rate of growth of the animals, the nutritive value of the samples were compared.

(2) The order of the nutritive value of the substances tested is as follows : As to Polysaccharide and Disaccharide, Maltose is most effective, followed by Starch, Dextrin, Lactose, Saccharose, Inulin, Glycogen and Funkan, and Konjak is far inferior to them and Agar-Agar is entirely invalid.

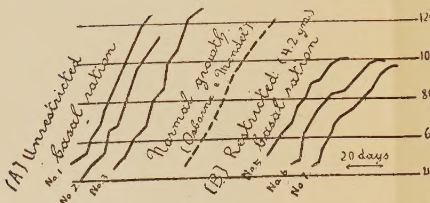
About Monosaccharides, Fructose and Glucose are most effective as well as Starch and Galactose, Mannose, Arabinose and Xylose come next, and Dioxyacetone and *α*–Methylglucoside are least effective ; Glucosamine is noxious at the amount of 1.25 g. As to the effectiveness of Alcohols, Ethylalcohol seems to be most nutritive one and Glycerin follows it ranking with Starch, while Mannite is far inferior to the latter. Ethyleneglycol is poisonous, when fed with the amount of 1.25 g. of it.

As to the Acids, the nutritive value of Gluconic acid, Saccharic acid and

Chart I.

The increase of body weight

of rats on basal ration.



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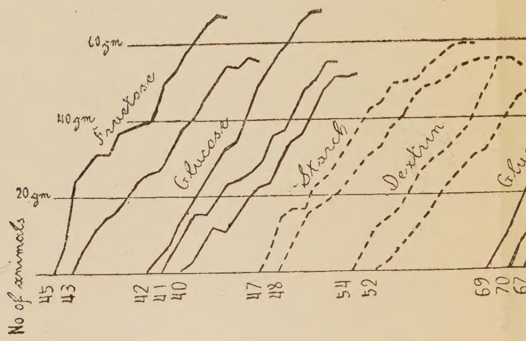

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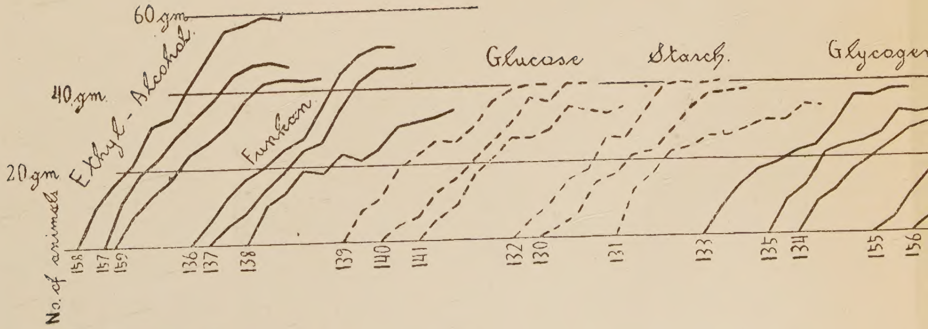
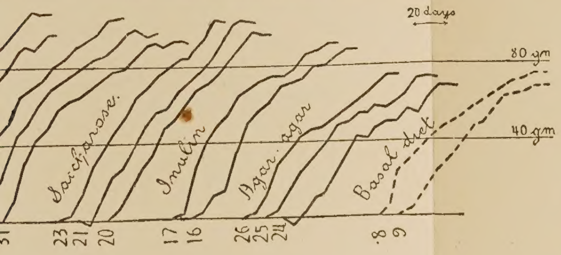
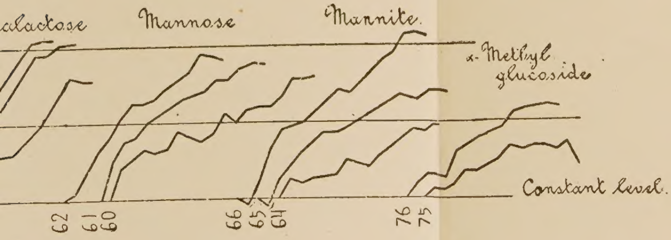


Chart II.

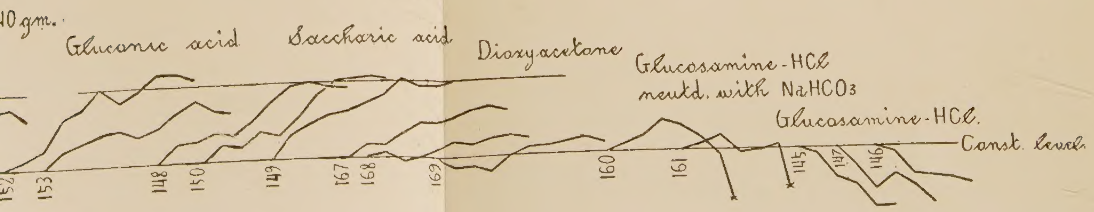
the increase of body weight of rats
es (Experiment I.).



r adding samples. (Experiment II.).



r adding samples. (Experiment III.).



Alanine is half of Glucose and Mucic acid, Glyceric acid and Phenylalanine are noxious at the amount of 1.25 g.

(3) Pentoses i. e., Arabinose and Xylose are found to be nutrive under our experimental condition.

(4) At the end of the feeding period, the urine of the animals was tested for reducing substances with Benedict's reagent and with the urine of Lactose, Galactose, Mannose, α–Methylglucoside, Glucosamine, Arabinose, Xylose and Dioxyacetone positive results were obtained.

The relatively low assimilability of these carbohydrates may be regarded as a cause of their low nutritive value. In spite of its low assimilability, Lactose was much effective on the growth of the animals, especially in the early part of the feeding period.

THE ASSIMILATION LIMIT OF CARBOHYDRATE

By

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(From the Agricultural Chemical Laboratory of Tokio Imperial University, Komaba.)

(Received Dec, 11 th., 1929).

As to the assimilability of carbohydrates, various studies have been reported by many investigators but they used mainly man, dog and rabbit as their experimental subjects.

In our previous experiment on the nutritive value of various carbohydrates, we observed that the assimilability of carbohydrates had a close relation to their nutritive value and in the present experiment, using the albino rats as the experimental animals, we measured assimilation limits of 13 kinds of carbohydrates.

Experimental method.

The experiments were carried out chiefly after the method of Mendel and Jones (J. Biol. Chem., **43**, 491, 1920.) who had measured the tolerance of various sugars with rabbits. The ration for the albino rats consisted of meat protein 15%, starch 70%, lard 10% and McCollum salt mixture 5%.

To each 12 g. of this mixture 0.5 mg. of Biosterin and 0.01 g. of the purified Oryzanin were added.

After feeding the rats with the ration above mentioned for about 10 days, the urine was collected and tested qualitatively for reducing substances with Benedict's reagent and if the reaction was negative the rats were submitted to the experiments.

20 male albino rats weighing about 150 g. were divided into four lots and they were used repeatedly for the experiments, being submitted one lot to the experiment of one kind of carbohydrate.

The definite dosage of carbohydrate to be tested was mixed with a small portion of the ration and was given to the rats and when this mixture was all eaten up by the animals, the remainder of the ration was supplied. After the rats ate sufficient enough amount of the diet they were divided into the wire cage provided with the funnel at the bottom and the urine voided during 18 hours was collected and tested with Benedict's reagent.

When the reaction was negative the amount of carbohydrate was increased 0.1 to 0.2 g. per 100 g. of body weight until the sugar tested appeared in the urine. According to Mendel and Jones the smallest amount of carbohydrate which resulted in glucosuria was recorded as the assimilation limit.

In the experiments of polysaccharides and disaccharides the urine was hydrolyzed with 1% HCl before it was tested for sugar.

In the experiment of Glucosamine we used Nylander's reagent in stead of Benedict's reagent, for the urine of the animals fed with Glucosamine-HCl -salt contained always reducing substance (or substances) other than the suger. As to the mixture of carbohydrates, the two components were mixed in the proportion of 1 : 1.

Results of the experiments.

The results of the expriments were shown in Table I, II, III, and IV. The urine of the animals fed with Inulin and Dextrin reduced the reagent only after the inversion and so these two carbohydrates are seemed to be excreted in the non-reducing polysaccharide form. Saccharose may be regarded as to be excreted in the unchanged-or inverted form, because out of five animals the urine of three animals (No. 101, 102, 103.) reduced the reagent while the urine of the other two animals (100, 104.) exhibited the reducing action after inversion. The comparison of our results with that of Mendel and Jones is given in the table V. As is shown in this table, the two figures for the tolerance of Maltose, Glucose, Saccharose and Fructose are almost similar, except that the order of Saccharose and Fructose is reversed.

As for Dextrin our average figure is 0.82 g., corresponding to half that of Mendel and Jones (1.61 g.)

In our results the assimilation limit of the mixture of sugars lies between that of two components while in the results of Mendel and Jones the tolerance for the mixture is less than that of either component (excepting for the Dextrin–glucose mixture.)

TABLE I.

Animal No.	Limits of assimilability per 100 g. of body weight.		
	Galactose	Lactose	Glucose + Lactose
	g.	g.	g.
90	−0.10	0.30	0.20
91	0.10	0.10	0.20
92	0.10	0.10	0.30
93	−0.10	0.10	0.40
94	0.10	0.20	0.20
average tolerance	−0.10 or 0.10	0.16	0.26

(Minus signs indicate that the assimilation limit was less than figure given).

TABLE II.

Animal No.	Limits of Assimilability per 100 g. of body weight.							
	Xylose	Arabinose	Fructose	Saccharose	Saccharose + Dextrin	Saccharose + Maltose	Fructose + Glucose	Dextrin + Maltose
	g.	g.	g.	g.	g.	g.	g.	g.
100	−0.10	0.10	0.40	0.80	0.80	1.10	1.00	1.40
101	″	″	0.50	0.70	0.80	1.00	0.90	1.40
102	″	″	0.60	0.80	0.70	1.20	1.00	1.20
103	″	″	0.40	0.70	0.70	0.70	1.00	1.20
104	″	″	0.60	0.80	0.80	0.90	1.00	1.40
average tolerance	−0.10	0.10	0.50	0.76	0.76	0.98	0.98	1.32

TABLE III.

Animal No.	Limits of Assimilability per 100 g. of body weight.						
	Mannose	Dextrin	Saccharose + Glucose	Dextrin + Glucose	Glucose	Maltose	Inulin
	g.	g.	g.	g.	g.	g.	g.
110	0.20	0.80	1.00	0.90	1.10	1.50	1.50
111	0.20	0.80	0.80	0.90	1.00	1.40	1.50
112	0.30	0.90	0.80	1.00	1.10	1.60	1.50
113	0.30	0.80	1.20	1.00	0.90	1.60	1.60
114	0.40	0.80	1.00	1.00	1.10	1.40	1.70
average tolerance.	0.28	0.82	0.96	0.96	1.04	1.50	1.56

TABLE IV.

Animal No.	Limits of Assimilability per 100 g. of body weight.	
	Glucosamine HCl-salt	Dioxyacetone
	g.	g.
120	0.30	0.30
121	0.20	0.20
122	0.40	0.40
123	0.30	0.30
124	0.30	0.40
average tolerance	0.30	0.32

TABLE V.

Carbohydrates.	Average tolerance per 100 g. of body weight.	
	The authors	Mendel and Jones
	g.	g.
Inulin	1.56	——
Maltose	1.50	1.71
Glucose	1.04	1.30
Dextrin	0.82	1.61
Saccharose	0.76	0.75
Fructose	0.50	0.80
Dioxyacetone	0.32	——
Glucosamine HCl-salt	0.30	——
Mannose	0.28	——
Lactose	0.16	——
Arabinose	0.10	——
Galactose	−0.10 or 0.10	——
Xylose	−0.10	——
Dextrin + Maltose	1.32	1.50
Saccharose + Maltose	0.98	——
Fructose + Glucose	0.98	0.75*
Saccharose + Glucose	0.96	——
Dextrin + Glucose	0.96	1.46
Saccharose + Dextrin	0.76	0.69
Lactose + Glucose	0.26	——

* Mendel and Jones studied with inverted Saccharose.

ON THE OXIDIZING ACTION OF CRYPTOMERIA-TIMBER, VESSELS OF SAKÉ AND SHŌYU.

By

MASAKAZU YAMADA

(Received Dec. 12 th., 1929.)

The properties of the alcohol oxidizing power of Cryptomeria-timber(1) were further investigated. The same action is common to some other woods.

In Cryptomeria the zone between heart-and sap-wood is generally the most powerful, the heart-wood moderate and the outer part of sap-wood, the leaf, the bark and the twig are far inferior whatever the unseasoned or not. The color of alcohol solution produced by the timber has not to do with the activity. The optimal temperature for the activity is 25 to 50°C. The timbers steamed for half an hour at 100° and also heated in the dry state for an hour at 150° lose the power. As to the effect of the reaction at PH below 9.0 the activity is gradually reduced, above which no remarkable influences being perceived. The lost or weakened action by heating or by the treatment of alkali is no more recovered. In the medium removed oxygen, the power is also reduced but recovered in this case by supply of the air. Only in the anhydrous alcohol the formation of aldehyde is quite restricted. The period of steeping is worthy of care and once practically aldehyde produced in 15% of alcohol solution reached 0.57% after 2 years.

The origin of oxidation is hardly ever separated from the timber with several solvents——water, alcohol, ether, weak acid solution, toluene-water, chloroform-water, petroleum-ether, 50% of glycerine and acetone——and the residue is always active. The oxidizing power is not due to the action of substances like aldehyde or ketone(2), because for those the sunlight is indispensable while in the timber oxidation takes place smoothly even in the cold, dark place. Moreover the action is not the conduct of either terpene(3), oxidase or peroxidase, for the resin of pine-tree or Cryptomeria, terpene oil, flesh potato and wheat-bran have all almost no effect upon the alcohol solution although the reaction of peroxide for KI solution or those of the oxidizing enzymes for guajac tincture etc. were evidently shown. Thus one of the oxidizing enzymes 'dehydrase'(4) or in a wider sense some oxidizing catalysts may be appointed as a main cause of the oxidation. Lately T. Higashi(5) discovered the action in the rectified Cryptomeria oil, a sesquiterpene and designated it as a main cause. This explanation is very convenient for many

Coniferaes and perhaps it is so in some cases but embarrassed in the cases of persimmon, prunus etc. Moreover we see that the action of terpenes is not so strong as the timbers.

Experimental.

In every case ca. 15%[×] alcohol solution with 3.5 g. of chips of timber for each 100 c.c. of the solution enclosed in a bottle was used and aldehyde was estimated after 14 days at ordinary temperature.

1. Aldehyde formation with several timbers continued.

Pine 0.02804 g. Chamaecyparis obtusa 0.01300 g. Crape-myrtle 0.00228 g. Chestnut 0.00170 g. Plum 0.00205 g. Persimmon 0.00448 ; 0.00887 g. Apple 0.00124 g. Mulberry 0.00151 g. Fatsia japonica 0.00232 g. Fig 0.00097. Maple 0.00206 g. Oak 0.00159 g. Cercidiphyllum japonicum 0.00136 g. Zelkova acuminata 0.00182 g. Ebony 0.00410 g. Olegon-pine 0.02241 g. White cedar 0.00354 g. Abies firma 0.00164 g. Celtis sinensis 0.00094 g. Prunus communis 0.00968 g. Cherry 0.01091.

Pomegramate, Gingko, Sultan's parasol, Camellia, Thea, Cape-jasmine, Rose-wood, Western red cedar, White beech, Mahogany, Red cedar, Wall-nut, Lignumvitae, Serayah, Cork, Liquorice, Pasania cuspidata, Willow, Elaegmus pungens had no effect.

2. The acition of the different positions. (Following data are all with Cryptomeria.)

(i) Bark (fresh), Twig (fresh), Inactive ; Leaf (fresh) 0.00087 g. Leaf (dry) 0.00391.

		I	II	III	IV	V	VI
		g.	g.	g.	g.	g.	g.
(ii)	heart-wood	0.02894	0.02200	0.01158	0.00873	0.01559	0.00726
	middle part	——	——	——	0.01513	——	0.02037
	sap-wood	0.00145	0.01506	0.00232	0.00495	0.02604	0.01164

(iii) With timbers for storage pail or cask.

The middle part between heart-and sap-wood is said to be the most suitable for the demand of saké-brewing and as a rule white-wood (sap) is used outside.

	A		B		C	
	red wood side	white wood side	red	white	red	white
	g.	g.	g.	g.	g.	g.
after 14 days	0.01521	0	0.01304	0.00792	0.01400	0.01557
after 32 days	0.02380	0.00169	0.02546	0.02101	0.02463	0.03574
after 90 days	0.03976	0.00170	0.18012	0.08914	0.08925	0.08763

3. Influence of temperature.

i. The bottle was incubated for 2 weeks.

7°	20°	26°	30°	40°	50°
0.01621 g.	0.01772 g.	0.02681 g.	0.02479 g.	0.02850 g.	0.02378 g.

[×] average content of saké.

ii. With the timbers treated in hot water at following temperatures for 30 minutes.

60°	70°	80°	90°	100°	100°× (5 min.)	100°× (10 min.)	100°× (15 min.)
0.00897 g.	0.00717 g.	0.00299 g.	0.00119 g.	0	0.00642 g.	0.00257 g.	0.00174 g.

iii. With timbers treated in the dry state.

100° 5 h.	110° 1 h.	120° 1 h.	130° 1 h.	140° 1 h.	150° 1 h.
0.00996 g.	0 00648 g.	0.00594 g.	0.00324 g.	0.00270 g.	0

4. In anhydrous alcohol. Aldehyde formed 0.00146%.

5. The effect of the reaction.

Pʜ 2.2	3.0	6.0	7.0	8.4	9.8	Na_2CO_3 1g.	Na_2CO_3 1.5g.
0.01415 g.	0.01938 g.	0.01179 g.	0.01703 g.	0.01257 g.	0.00917 g.	0.00419 g.	0.00367 g.

6. Removal of oxygen with alkaline pyrogallol solution.

	A	B
Removal (after 14 days)	0.00669 g.	0.00690 g.
Supply (after 53 days)	0.02275 g.	0.01600 g.

7. With the extracts and residues of timbers.

Solvent	Ether	Water	Alcohol (73%)	Pet.-ether	1% HCl	1% Lactic	$CHCl_3$ 2 c.c. water 81 c.c.	Toluene 1 c.c. water 82 c.c	Glycerine 50%	Acetone, ether, alc.
Period of extraction	3 days	35 days	35 days	2 days	12 days	12 days	2 days	2 days	2 days	2 days
With the extract	0.00428 g.	0.00136 g.	0.00156 g.	——	0	0	0	0	0	—
With the residue	0.04203 g.	——	——	0.01469 g.	——	——	0.01335	0.01643	0.01971	0.00478
Period of steeping	32 days	35 days	35 days	14 days	14 days	14 days	14 days	14 days	14 days	14 days

8. Comparison of oxidizing action of timber with that of aldehyde or ketone (p-benzo-chinone as a representative).

	Treatment	Color of sol.	Aldehyde	Remarks
(1)	Timber 35 h. under direct sun light	brown	0.01273 g.	no special increase
(2)	Chinone 0.5 g.	black	0.06299 g.	Riminis react. blue
(3)	Chinone 14 days in the dark place	greyish brown	0.00256 g.	
(4)	Camphor 1.5 g. 〃	Colorless	0	
(5)	Hydrochinone 0.5 g. 〃	Yellow	0	
(6)	Pyrogallol 0.5 g. 〃	Pale brown	0	

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(3) Waksman and Davison: Enzymes p. 230 (1926).
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(5) T. Higashi: Bull. Inst. Phys. Chem. Research, **8** 97, (1929).

(From the Imperial Brewing Experimental Station, Takinogawa-machi near Tokyo.)

× 5% alcohol solution.

ON VITAMINS IN SAKE-CAKE.

III. ON ERGOSTERIN IN SAKE-CAKE.

By

EIJI TAKAHASHI and H. LIM.

(Received Dce. 15 th., 1929).

The present paper deals with the separation of Ergosterin from Sake-Cake and Koji-fungus (Aspergillus Oryzae).

1 kg. of dried Sake-Cake was extracted with 85~95% alcohol at 80°C. The extract was evaporated and treated with ether. The substance soluble in the later solvent was separated and refined with acetone. 125 g. of oily substance thus obtained was next saponified with alcoholic potash solution and the unsaponifiable part was taken with ether. By evaporation, Sterin-substance which made of needle and plate like crystals was obtained. The melting point showed 137°C.

As it was considered from the result that the separated substance contained the other sterin having higher melting point than phytosterin, the biological test was next executed.

Albino rats was taken, Steenbock's No. 2965 was given as basal diet, the symptom of ricket was diagnosed by lentogen. When the rats were fed for 5 weeks with above diet, they indicated the characteristic pathological change at the end of shinbone. But by feeding the isolated crystals which were formerly irradiated, cured or prevented the disease. 1/500 mg. of the crystal was able for prevention and 1/50 mg. for cure.

The irradiation was done as follows:

The sample was dissolved in Petri-dish with ether, after evaporation was exposed to the light at the distance of 25 cm. for 40 minutes. The lamp was such small type as passing 2 ampere, 90~100 volt.

Further the separation of ergosterin from the Koji-fungus which was cultivated in the solution free from organic substance but sugar, was tried and the animal experiment was also done. By the same method as above 1.25 g. needle crystals were obtained from 500 g. sample. Its melting point was 163~165°C. $[\alpha]_D^{18} = -126°$, these agree well with ergosterin.

The analytical result was as follows:

32.36 mg. sample gave CO_2 100.33 mg. H_2O 31.89 mg.

	C	H
Calculated for $C_{27}H_{42}O$	84.75%	11.07%
Found	84.55%	10.95%

The experiment with rats showed 1/5000 mg. prevented the disease and 1/1000 mg. cured it after 3 weeks.

UEBER DIE OBERFLÄCHENAKTIVITÄT UND DIE ADSORBIERBARKEIT VON AMINOSÄUREN.

I. MITTEILUNG.

Von

TAKEO ITO.

(*Aus dem Agrikulturchemischen Laboratorium in Landwirtschaftlichen Hochschule, Morioka*)

(Angenommen, Jan. 12, 1930).

In den Untersuchungen über die Adsorption von Aminosäuren und Polypeptiden durch Tierkohle haben E. Abderhalden und A. Fodor(1) gezeigt unter anderem, dass sie in mitteleren Konzentrationen nach der gewöhnlichen Adsorptionsisotherme adsorbiert werden, während bei kleinen Konzentrationen das Henrysche Gesetz gilt. Sie nahmen, um die von ihnen beobachteten Tatsachen erklären zu können, an, dass die Adsorption von genannten Stoffen an Tierkohle kein rein physikalischer Vorgang, vielmehr chemischen Kräften zuzuschreiben sei.

Die vorliegende Untersuchung wurde unternommen, um zu pfrüfen, ob sich die wohlbekannte Beziehung zwischen der Oberflächenaktivität und der Adsorbierbarkeit von oberflächenaktiven Stoffen, wie z.B. Fettsäuren, auch bei Aminosäuren erkennen lässt.

(1) Die zur Untersuchung herangezogenen Aminosäuren wurden teils (Glykokoll, d-Alanin, l-Leucin und d-Glutaminsäure) nach Estermethode aus den sie reichlich enthaltenden Eiweisskörpern, teils (dl-α-Aminobuttersäure und dl-Valin) synthetisch aus den entsprechenden Fettsäuren hergestellt. dl-Asparaginsäure war ein Präparat von Merck („für wissenschaftliche Zwecke"). Ihre Reinheit wurde analytisch bzw. physikalisch-chemisch bestätigt.

(2) Die Oberflächenspannung der wässerigen Aminosäurelösungen bei verschiedenen Konzentrationen wurde bei $18 \pm 0{,}5°C$ mit Hilfe von Tropfenmethode gemessen, indem man ein Stalagmometer nach Traube benutzte. Die Ergebnisse finden sich in Fig. 1 und 2.

In oben stehenden Figuren ist die relative Oberflächenspannung σ, bezogen auf die des Wassers, die gleich 1 gesetzt wird, als Ordinate und die Konzentration c in Mol im Liter als Abszisse zugeordnet. Man ersieht aus Fig. 1, dass die Aminomonocarbonsäuren merklich weniger oberflächenaktiv sind als die entsprechenden Fettsäuren (vgl. hierzu Freudlich, Kapillarchemie, 3. Aufl. (1923)).

Glykokoll und Alanin erhöhen sogar die Oberflächenspannung des Wassers. Trotzdem findet sich die Regelmässigkeit auch hier statt, mit der die Oberflächenspannung mit dem Ansteigen in der homologen Reihe zunimmt. Wie aus Fig. 2 ersichtlich, sind Asparaginsäure und Glutaminsäure oberflächeninaktiv.

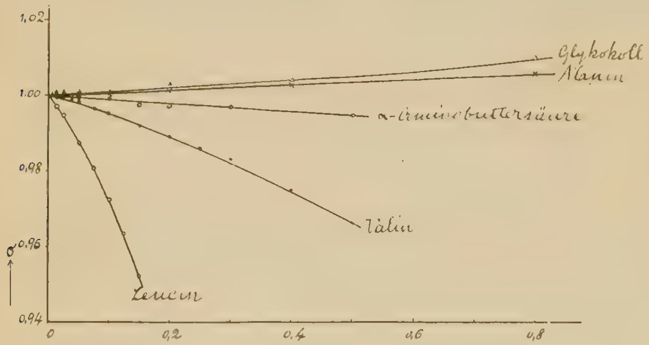

Fig 1
σ-c-Kurven von wäßrigen α-Aminomonocarbonsäurelösungen.

(3). Für Adsorptionsversuche verwendete ich Knochenkohle (Merck, Carbo animalis pur. sicc.), die bei 100 ∼110°C bis zum konstanten Gewichte getrocknet worden war.

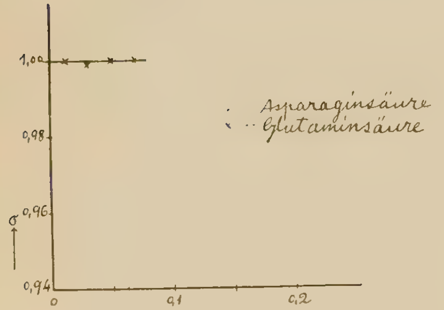

Fig. 2
σ-c-Kurven von wäßrigen α-Aminodicarbonsäurelösungen.

Ca. 1,25 g. Kohle wurden in einen kleinen mit eingeschliffenem Glasstopfen verschliessbaren Erlenmeyerkolben genau eingewogen und mit 25 ccm Aminosäurelösung, die vorher auf 18°C gebracht worden war, zusammengebracht. Nach 20 Minuten Schütteln im Thermostat bei 18±0,1°C wurde die Kohle abfiltriert, wobei die ersten ca. 5 ccm. Filtrat weggeworfen wurden. Der Aminosäuregehalt der Lösungen nach wie vor der Adsorption wurde nach Van Slyke bestimmt und aus der Differenz zwischen beiden Bestimmungen liess sich die adsorbierte Menge berechnen.

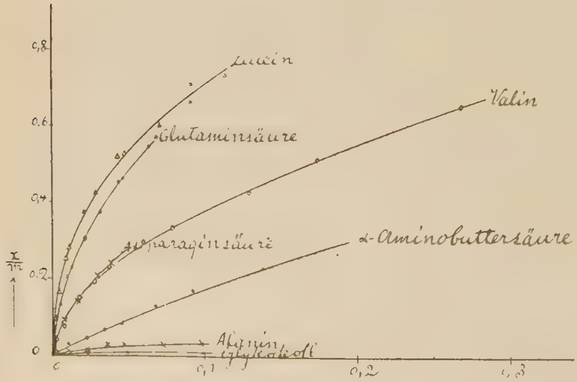

Fig. 3.
Adsorptionsisothermen von wäßrigen Aminosäurelösungen

Die Resultate sind in Fig. 3 graphisch dargestellt, wobei als Ordinaten m/x die adsorbierten Aminosäuremengen in Millimol pro g.

Kohle, als Abszissen c die Endkonzentrationen in Mol im Liter aufgetragen sind.

Es ist aus oben stehender Figur zu ersehen, dass die Aminosäuren durch Tierkohle geringer adsorbiert werden als die entsprechenden Fettsäuren. Vergleicht man Fig. 1 mit Fig. 3, so erkennt man einen deutlichen Parallelismus zwischen der Oberflächenaktivität und der Adsorbierbarkeit von Aminomonocarbonsäuren.

Zur näheren Orientierung sind in Tabelle I die aus Fig. 1 graphisch ermittelten m/x-Werte und die relativen Oberflächenspannungswerte σ ($\sigma_{H_2O}=1$) zusammengestellt, beide bezogen auf eine Gleichgewichtskonzentration von 0,1 Mol im Liter.

TABELLE I.

Aminosäure	Relative Oberflächenspannung σ bei $c=0{,}1$ Mol i. L.	Adsorbierte Menge m/x in Millimol pro g Kohle bei $c=0{,}1$ Mol i. L.
Glykokoll	1,001	0,01
Alanin	1,000	0,035
α-Aminobuttersäure	0,9989	0,17
Valin	0,995	0,39
Leucin	0,9722	0,71

Hieraus ersieht man, dass die in der homologen Reihe höher stehenden Aminosäuren merklich oberflächenaktiv und dementsprechend ziemlich gut adsorbierbar sind, und zwar in der Reihenfolge.

α–Aminobuttersäure $<$ Valin $<$ Leucin.

Bemerkenswert ist, dass Glykokoll und Alanin, die bei der in Betracht kommenden Konzentration die Oberflächenspannung des Wassers gar nicht erniedrigen, eher ein wenig erhöhen, durch Kohle aufgenommen werden, wenn auch in geringem Masse.

Asparaginsäure und Glutaminsäure werden, wie aus Fig. 3 ersichtlich, an Kohle merklich adsorbiert, obwohl sie, wie schon erwähnt, oberflächeninaktiv sind. Indessen ist ähnliche Erscheinung auch bei anderen Stoffen, wie z.B. Bernsteinsäure, einigen Zuckerarten usw., bekannt (vgl. Freundlich, loc. cit., S. 268).

Tabelle 2 und 3 enthalten die Ergebnisse der Adsorptionsversuche, wobei für 25 ccm. Aminosäurelösung 1,25 bzw. 1,24/4 g. Kohle angewandt wurden.

TABELLE II.

Adsorption von Leucin an Knochenkohle.

$$x/m = 1{,}87^{\circ}C^{\,0{,}422}$$

Kohlenmenge in g.	Anfangskonzentration in Mol i. L.	Endkonzentration c in Mol i. L.	Adsorbierte Menge in Millimol pro g. Kohle	
			x/m (beob.)	x/m (berech.)
1,2524	0,00625	0,0015	0,095	0,1203

0,3131	〃	0,0042	0,160	0,1858
1,2594	0,0125	0,0042	0,164	0,1858
0,3143	〃	0,0093	0,257	0,2597
1,2296	0,025	0,0112	0,281	0,2809
0,3094	〃	0,0203	0,376	0,3611
1,2570	0,05	0,0287	0,423	0,4149
0,3145	〃	0,0435	0,519	0,4981
1,2560	0,075	0,0484	0,529	0,5210
0,3127	〃	0,0678	0,571	0,6007
1,2510	0,1	0,0699	0,600	0,6084
0,3162	〃	0,0914	0,708	0,6813
1,2530	0,125	0,0918	0,661	0,6829
1,2548	0,15	0,1132	0,733	0,7457

TABELLE III.

Adsoption von Glutaminsäure an Knochenkohle.

$$x/m = 2{,}53 \, C^{0.554}$$

Kohlenmenge in g.	Anfangskonzentration in Mol i. L.	Endkonzentration c in Mol i. L	Adsorbierte Menge in Millimol pro g Kohle.	
			x/m (beob.)	x/m (berech.)
1,2536	0,00625	0,0026	0,072	0,0935
0,3112	〃	0,0040	0,098	0,1187
1,2510	0,0125	0,0060	0,129	0,1469
0,3109	〃	0,0100	0,204	0,1973
1,2562	0,025	0,0134	0,230	0,2320
0,3152	〃	0,0213	0,302	0,2999
1,2583	0,05	0,0313	0,372	0,3712
0,3124	〃	0,0444	0,455	0,4506
1,2537	0,07	0,0464	0,470	0,4617
0,3103	〃	0,0632	0,544	0,5477

In oben stehenden beiden Tabellen sind neben der experimentell ermittelten Grösse x/m die nach der an der Spitze der betreffenden Tabelle angeführten Formel berechneten x/m-Werte angegeben. Die experimentell ermittelten und die berechneten Werte sind sehr nahe. Mit anderen Worten: Bei Leucin bzw. Glutaminsäure–und ähnliches dürfte auch für die anderen Aminosäuren gelten–lässt sich die Adsorption, unabhängig von der angewandten Kohlenmenge, je durch eine Adsorptionsisotherme wiedergeben.

LITELATURE.

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Kolloid Zeitschr: 27, **49** (1920).

STUDIES ON BACILLUS THERMOFIBRINCOLUS N. SP. III.

PRODUCTION OF VOLATILE ACID AND ALCOHOL.

By

ARAO ITANO and SATIYO ARAKAWA

(Received Jan. 20 th., 1930).

The results of further investigation on B. thermofibrincolus n. sp. as to the production of volatile acid and alcohol together with the quantitative determination of cellulose fermented, are reported below:

(1) In course of cultivation of the organism in a test tube at 65°C., 45.5% evaporation takes place in a week. But it can be prevented by placing an inoculated tube in another larger tube with a cork stopper which has small holles.

(2) More cellulose is fermented in a test tube than that in a 300 c.c. flask although more volatile acid and alcohol are produced in the latter. At 65°C, the organism ferments 60～88% cellulose supplied at the beginning, in 3～7 days, and produce 24～33% volatile acid, as acetic acid and 3～12% alcohol, as etheyl alcohol in proportion to the quantity of cellulose fermented.

(3) The optimum temperature for the organism is 65°C., and at 50°C. ferments only 63% cellulose as to that fermented at 65°C. But the amount of volatile acid and alcohol produced is rather large at 50°C., comparing to that at 65°C. This may be due to the facts that some of the products are lost at the higher temperature and also used up in the metabolic process.

(4) As to the influence of oxygen, less quantity of cellulose is fermented under anaerobic condition (Buchner's method) than that under aerobic condition. Although a similar amount of volatile acid is produced in both cases, more than the double amount of alcohol is produced under the anaerobic condition.

(5) The influence of different concentration (0.0～2.0%) of peptone was determined and found 0.5% is the optimum, and the less than the optimum amount gives better fermentation than that at higher concentration. Even without an addition of peptone, fairly good fermentation takes place. The quantity of volatile acid produced parallels the quantity of peptone given.

(6) Different amount of cellulose has different influence; 0.75～2.20% inclusive is the best for both cellulose fermentation and volatile acid production but for alcoholic production, the larger amount of cellulose is better.

(7) As to the influence of nitrogen source, the following relations were found:

For cellulose fermentation;

peptone > egg albumin > casein > ammoniun sulfate > meat extract.

For volatile acid production;

meat extract > egg albumin > peptone > casein > ammonium sulfate.

For alcoholic production;

meat extract > peptone > egg albumin > casein > ammonium sulfate.

Thus the organism seems to require the organic nitrogen for both production of volatile acid and alcohol.

(8) In the medium which contains $CaCO_3$, 64% cellulose fermented on the third day while only 32%, without $CaCO_3$: on the seventh day, 88% fermented in the former and 46% in the latter. The production of both the volatile acid and alcohol is greatest on the third day. Such marked difference of fermentation seems to be due to the accumulation of organic acid which interferes with the process where no $CaCO_3$ is present. Again on this basis, it is possible to follow the physiological activity of the organism by determining the pH values.

STUDIES ON BACILLUS THERMOFIBRINCOLUS N. SP. VI.

NITROGEN METABOLISM.

By

Arao Itano and Satiyo Arakawa

(Received Jan, 20th., 1930).

The action of the organism as to the transformation of amino and ammonia nitrogen in the culture medium with and without an addition of cellulose.

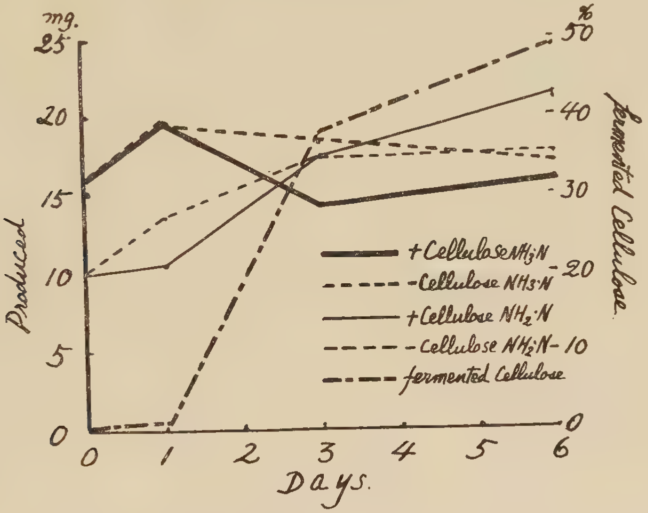
The results are shown in the following figure; Fig. I.

As Fig. I. indicates, in presence of cellulose, the quantity of ammonia nitrogen produced is irregular while the amino nitrogen increased steadily. In case no cellulose is present, both ammonia and amino nitrogen decreased after certain days. Consequently in presence of cellulose, the quantity of amino acid can serve as an approximate index of proteolysis, and it seems to

indicate that the organism contains the proteolytic enzyme. This confirms the experimental results obtained by De Bord.

Fig. I.

Nitrogen Metabolism.



UEBER DAS VORKOMMEN VON SCHWEFLIGER SAEURE IN VERGOERENER FLUESSIGKEIT.

Von

Mosuke Matsuyama und Hiroichi Nakamura

(Aus dem Laboratorium der Union-Brauerei bei Tokyo, Japan.)

(Angenommen Jan, 25, 1930).

Obgleich wir in der vorhandenen Literatur mehrfach ueber Versuche mit Schwefelwasserstoff in vergorener Fluessigkeit gelesen haben, haben wir bisher nichts gefunden ueber die schweflige Saeure, die auch stets in kleinen Mengen in vergorener Fluessigkeit, z.B. in Bier und Wein, enthalten ist. Wir haben Untersuchungen auf diesem Gebiet angestellt und teilen die Resultate in folgendem mit:

Nach einigen Vorversuchen wiesen wir das Vorhandensein von schwefliger Saeure wie folgt nach:

Zu 200 ccm der Probe fuegt man 10 ccm von 25%iger Phosphorsaeureloesung hinzu und destilliert unter Durchleitung von durch Kaliumpermanganat-Loesung und Wasser gewaschener Kohlensaeure, bis 100 ccm Destillat gewonnen ist. Als Vorlagekolben dient ein Erlenmeyer'scher Kolben, welcher 20 ccm Jod-Jodkalium-Loesung (5 g. I und 7,5 g. KI in 1 ltr. Wasser geloest) enthaelt. Man setzt dem Destillat 10 ccm konzentrierte Salzsaeure hinzu und erhitzt es, bis die Jodfarbe verschwindet. Dann bestimmit man die Schwefelsaeure als Bariumsulfat durch Zusatz von 20 ccm 10%iger Bariumchloridloesung.

Zunaechst wurd die schweflige Saeure im Rohstoff bestimmit. Die Probe wurde mit 200 ccm Wasser und 0,5 g. Sodiumcarbonat 6 Stunden extrahiert, und die Auszuege wurden in der oben angegebenen Weise behandelt. Wir stellen die Resultate in folgender Tabelle zusammen:

Rohstoffe	Probe (g.)	Schweflige Saeure (g.) (als $BaSO_4$)	Schweflige Saeure in 100 g. Substanz
Malz I (direkt gedarrt)	30	0,0018	0,0060
Malz II (indirekt gedarrt)	30	0,0007	0,0023
Hopfen (Saazer I)	10	0,0071	0,0710
Hopfen (Saazer II)	10	0,0162	0,1620
Hopfen (amerikanisch)	10	0,0428	0,4280
Reis	30	0,0010	0,0033

Wir haben sodann mit 270 g. Malz II, 80 g. Reis, 6 g. Hopfen (Saazer II) und Wasser 2 ltr. den Wuerze gemaischt und die schweflige Saeure derselben bestimmit. Nach einer auf Grund der gebrauchten Rohstoffe angestellten Berechung haetten 100 ccm Wuerze 0.0009 g. schweflige Saeure (als $BaSO_4$) enthalten muessen; wir fanden jedoch, dass die Wuerze nur geringe (0,0001 in 100 ccm) und nicht genuegend bestimmbare Mengen enthielt. 700 g. Malzschrot wurden mit 3,5 ltr. Wasser gemaischt, mit 10,5 g. Saazer Hopfen gehopft, und nach einstuendigem Kochen wurde die Wuerze durch Zusatz von Wasser geeicht, sodass sie einem Extrakt von 12,5° Balling entsprach. Zum Maischen wurden verschiedene Arten Wasser gebraucht und verschiedene Sorten Wuerze gewonnen. 500 ccm der Wuerze wurden in Kolben verteilt, sterilisiert und mit Bierhefe vergoren in einem Zimmer mit einer Temperatur von 10~12°C (Hierfuer wurden zwei verschiedene Staemme von untergaeriger Hefe unserer Brauerei benutzt, welche wir mit A und B bezeichnen). Vor und nach der Gaerung wurde die schweflige Saeure bestimmt. Die Resultate sind aus folgenden Tabellen ersichtlich.

Wuerze mit destilliertem Wasser:

	Vergaerungstage	Probe (ccm.)	SO_2 (g.) (als $BaSO_4$)	〃 in 1 ltr.	〃 Zunahme
Wuerze	0	200	0	0	

Vergoren mit Hefe A	9	200	0,0012	0,0060	0,0060
〃 〃 〃 B	9	200	0,0012	0,0060	0,0060

Wuerze mit Brunnenwasser:

Wuerze	0	200	0,0002	0,0010	
Vergoren mit Hefe A	9	200	0,0012	0,0060	0,0050
〃 〃 〃 B	9	200	0,0010	0,0050	0,0040

Wuerze mit Magnesiumsulfat-Wasser (0,28 g. $MgSO_4$ in 1 ltr.):

Wuerze	0	200	0,0004	0,0020	
Vergoren mit Hefe A	9	200	0,0012	0,0060	0,0040
〃 〃 〃 B	9	200	0,0022	0,0110	0,0090

Wuerze mit Gips-Wasser (0,28 g. $CaSO_4 \cdot 2H_2O$ in 1 ltr.):

Wuerze mit Gips-Wasser (0,34 g. $CaSO_4 \cdot 2H_2O$ in 1 ltr.):

Wuerze	0	200	0,0002	0,0010	
Vergoren mit Hefe A	9	200	0,0012	0,0060	0,0050
〃 〃 〃 B	9	200	0,0056	0,0280	0,0270

Wuerze mit Gips-Wasser (0,25 g. $CaSO_4 \cdot 2H_2O$ in 1 ltr.):

	Vergaerungstage	Scheinbarer Extrakt (Balling)	Probe (ccm)	SO_2 (g) (als $BaSO_4$)	〃 in 1 ltr.	〃 Zunahme
Wuerze	0	12,5	200	0,0002	0,0010	
Vergoren mit Hefe B	3	11,0	200	0,0008	0,0040	0,0030
	9	6,5	200	0,0036	0,0180	0,0170

Zusammenfassung.

(1) Die gehopfte und gekochte Wuerze aus Malz und Reis hat einen geringen Gehalt an schwefliger Saeure, welcher sich vergroessert durch Gaerung mit Bierhefe.

(2) Die Bildung von schwefliger Saeure bei alkoholischer Gaerung steht in naher Beziehung zu den Hefestaemmen und der Art und Menge des Sulfats in der Fiuessigkeit.

UEBER DIE VERTEILUNG VON PHOSPHORSAEURE IN GERSTE UND MALZ.

Von

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(Aus dem Laboratorium der Union-Brauerei bei Tokyo, Japan.)

(Angenommen Jan. 25, 1930).

Ueber die in Gerste und Malz vorkommende Phosphorsaeure liegen bereits aus anderen Laboratorien viele Untersuchungen vor, deren Resultate jedoch in einigen Punkted voneinander abweichen. Wir haben ebenfalls Untersuchungen ueber die Verteilung von Phosphorsaeure in Gerste und Malz vorgenommen. Als Untersuchungsmaterial diente in der Tochigi-Gegend im Jahre 1928 geerntete "Goldenmelon" und von derselben Gerste gekeimtes Malz. 20 g. von jeder Probe wurde zunaechst mit Aether und nacheinander mit Alkohol, 0.2% Salzsaeure und 10% Ammoniak extrahiert und die Phosphorsaeure—die Gesamtmenge und noetigenfalls auch die Menge von organischer und anorganischer Form—in jedem Auszug bestimmt. Weiterhin wurden 20 g. Proben mit 0.2%iger Salzsaeure extrahiert, und mit dem Auszug wurde die Phytin–Phosphorsaeure durch Faellung mit Kupferracetat–Loesung und weitere Behandlung mit Schwefelwasserstoff, Salzsaeure und Baryta bestimmt. Es wurde auch das Vorhandensein von Phosphoproteid–Phosphor, welcher durch Pepsin–Salzsaeureloesung (1% Pepsin in 0.2% HCl) ausgezogen wurde, bestimmt. Die Bestimmung der Phosphorsaeure wurde vorgenommen nach dem Verfahren von Lorenz, und die anorganishe Phosphorsaeure wurde bestimmt nach der Methode Schulze–Castro. Bei unseren Untersuchungen kamen wir zu folgenden Resultaten, wobei die Einzelergebnisse im Verhaeltniss zu $P_2O_5=100$ umgerechnet sind.

	Gerte		Malz	
	in Trockensubst.	% von Gesamt-P_2O_5	in Trockensubst.	% von Gesamt-P_2O_5
Gesamt-Phosphorsaeure	0,8570	100	0,8342	100
Phosphatid–P_2O_5	0,0987	11,52	0,0805	9,65
Anorganische–P_2O_5	0,1778	20,75	0,2452	29,39
Phytin–P_2O_5	0,0450	5,25	0,0408	4,89
Nuclein–P_2O_5	0,4915	57,35	0,4570	54,80
Phosphoproteid und unbestimmte–P_2O_5	0,0440	5,13	0,0107	1,27
	0,8570	**100**	**0,8342**	**100**

In der Tabelle zeigt die Phosphatid-P_2O_5 die Summe der in Aether und Alkohol loeslichen Phosphorsaeure, die anorganische die beim sukzessiven Extrahieren in 0.2%iger Salzsäureloesung loesliche, nach dem Verfahren von Schulze-Castro bestimmbare P_2O_5, und die Nuclein-P_2O_5 die in 10%igem Ammoniak loesliche Phosphorsaeure. Phytin-P_2O_5 wurde einzeln in der oben beschriebenen Weise bestimmt. Die Differenz zwischen der Gesamt-P_2O_5 (welche mit Asche von 3 g. Proben direkt bestimmt wurde) und der Summe von gegebenen anderen P_2O_5 bezeichneten wir als Phosphoproteid–und unbestimmte P_2O_5.

Aus unseren Ergebnissen ist ersichtlich, dass der Gehalt an organischen Phosphorsaeuren bei Malz geringer ist als bei Gerste, waehrend es sich mit anorganischen Phosphorsaeuren umgekehrt verhaelt. Hieraus erklaert sich die Wirkung von Phosphatasen waehrend der Keimung.

EFFECT OF STANNOUS CHLORIDE ON THE ACID HYDROLYSIS OF PROTEIN AS A PREVENTIVE AGENT FOR THE FORMATION OF "MELANIN SUBSTANCE".

By

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(Received Jan, 10 th., 1930).

The so-called "humin" or "melanin substance", darkly coloured and of unknown chemical constitution, are formed when proteins are boiled with mineral acid. Though our knowledge about the chemical nature of this substance is yet very limited, we can trace the origin of this substance which consists of some special amino acids, tyrosine, cystine, tryptophane and hexon bases on the one hand, and the carbohydrate accompaning proteins such as glucose or glucosamine on the other.

In the analytical courses of protein hydrolysate, the "melanin substance" makes many disadvantages about the accurate estimation of amino acids, therefore the devices to prevent the formation of "melanin substance" on the acid hydrolysis of protein were made, and Hlawetz and Haberman were the first who employed stannous chloride for this purpose. Some investigators followed this indication, but none payed attention about its applicability to the quantitative analysis of amino acids. Then the above matters were tested:

(1) By application of $SnCl_2$ to the nitrogen distribution analysis of the Van Slyke's method. The effect of $SnCl_2$ on hydrolising casein only and on hydrolising casein with glucose was as in results belows:

	Casein only (3 g.)				Casein (3 g.) with glucose (1 g.)			
	Usual method		$SnCl_2$ method		Usual method		$SnCl_2$ method	
Melanin-N.	1.40%	1.34%	1.43%	1.38%	2.07%	2.20%	2.73%	2.84%
Amide-N.	10.80	10.12	9.35	9.44	11.25	11.48	10.54	10.71
Diamino-N.	24.93	27.31	27.12	26.97	26.18	24.78	26.50	26.52
Arginine-N.	7.90	7.87	8.86	8.67	8.33	8.07	8.85	8.59
Histidine-N.	2.99	6.29	5.43	6.29	4.67	3.81	5.08	5.66
Cystine-N.	1.05	1.08	1.05	1.08	1.08	1.05	1.05	1.02
Lysine-N.	12.99	12.06	11.76	11.23	12.10	11.85	11.42	11.23
Monoamino-N.	56.46	55.14	54.86	55.69	53.96	55.51	55.17	55.63

Nonamino-N.	6.51	6.11	5.62	5.86	8.15	7.68	6.94	6.26
Total-N. recovered	**100.10**	**100 02**	**98 38**	**99.34**	**101·61**	**101·65**	**101.88**	**101.96**

Note: (1) The value of melanin-N. include:
a) N. in the darkly colored insoluble residue.
b) N. adsorbed with $Ca(OH)_2$ or tin sulphides.
c) N. in the phosphotungstate melanin.
(2) At the above method with $SnCl_2$ the filtrate from tin sulphide is completely colourless so the precipitate of phosphotungstates are mostly snow-white.

Judging from the colour of hydrolysate, the melanin formation is greatly reduced when $SnCl_2$ is added, but the apparent high values of melanin nitrogen at the side of $SnCl_2$ method may be attributed to the greater adsorbtion capacity of tin sulphides than that of $Ca(OH)_2$. The each value in the fraction of phosphotungstate precipitates on the method with $SnCl_2$ coinsides closely together in parallel analysis, and they do not alter when casein is hydrolised with the addition of glucose.

(2) The effect by addition of $SnCl_2$ on the formation of "melanin substance" when amino acids and carbohydrates are boiled together with concentrated hydrochloric acid is as follows: the amino acids, containing 0.05 g. of nitrogen, and 0.1 g. of glucose, were boiled with 100 c.c. of 25% HCl for 5 hours with or without the addition of 1 c.c. of 5n. $SnCl_2$ solution.

In general, regarding the "insoluble melanin substances": their colours, appearances, rough amounts and nitrogen contents, are observed, and as to the soluble forms the colours of solution and nitrogen adsorbed with tin sulphides when the metal is removed by H_2S are also noted. But as for the difference of adsorption capacities of SnS as well as SnS_2, this nitrogen values do not indicate the amount of soluble forms.

Glycocoll, leucine, glutaminic acid, histidine, arginine and ammoninm chloride gave no or little "melanin substances" cotaining nitrogen. On the contrary tyrosine, cystine and tryptophane gave much "melanin substances", but the addition of $SnCl_2$ gave much or less diminishing effect on melanin formation. By increasing the amount of glucose from 0.1 g. to 0.5 g., the added $SnCl_2$ became deficent.

Nextly, several kinds of carbohydrate and HCl were heated with or without the addition of $SnCl_2$. Glucose and fructose behaved likely, both producing black insoluble powdery substances, and slightly brown filtrate on the control experiment, and dark brown tallowy residue and faint yellow filtrate on the addition of $SnCl_2$. Arabinose also behaved almost likely with the formor cases, but a little different in producing the dark brown powdery "insoluble melanin substances" on the addition of $SnCl_2$. It is quite different in the case of furfurol, the addition of $SnCl_2$ had no effect on reducing the amount of insoluble black melanin which was produced immediately after the mixtures were heated. By this phenomena it is indicated that the added $SnCl_2$ makes its effect at the stage of condensation, sugars into the furfurol like-substances. In fact, if $SnCl_2$ is added the quantity of the furfurol produced from arabinose by the method of pentose estimation, diminished to about one half of the normal value. As to glucosamine the melanin formation is completely prevented by $SnCl_2$, and from mannit no melanin is formed at all.

The next experiment is the heating of amino acids: tyrosine or cystine, or tryptophane, with arabinose or glucosamine or fructose. In this case it is interesting that glucosamine is a very weak melanin producer, that is to say, no insoluble melanin is formed in all cases, also, the soluble formes produced from tyrosine or cystine are almost negligible, and only from tryptophane the dark brown solution is made when no $SnCl_2$ is added, but the colour doesn't appear when the reagent is added.

Throughout this experiment the attempt to express the amount of "melanin substances" by the nitrogen values was in vein, because the tin sulphides, on removing the metal› adsorbed not merely the melanin but also other nitrogeneous compounds. By experiments it was found that arginine, histidine, cystine and tryptophane were especially much adsorbed by stannic sulphide (SnS_2).

ON THE CHANGES OF CYANAMIDE IN THE SOIL. PART I.

A COMPARATIVE STUDY IN DRY-FIELD AND PADDY-FIELD CONDITIONS.

By

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(Received Dec. 14th., 1929).

Cyanamide, when applied to the paddy-field, has been found to destroy the icterohaemorrhagic spirochaeta in the soil (Tōyama, 1918). The application of commercial cyanamide to the paddy-rice field gives the good results for prevention of Spirochaetosis icterohaemorrhagicae (so-called Weil's disease), as well as the good fertilizing effect on the rice crop. Consequently, the direct use of commercial cyanamide in the paddy-rice field, prior to the transplantation of paddy-rice, is gradually spreading. Furthermore, the use of commercial cyanamide in various crop cultures is recently becoming more common in Japan, because of its relative cheapness as a nitrogenous fertilizer and its weed-killing and soil pests destroying power. Little, however, is known of the transformation of cyanamide in the paddy-field state. The present work was therefore undertaken to compare the changes of cyanamide nitrogen in two different soil conditions, namely dry-field (normal soil) and paddy-field (water-logged soil).

Methods.

The several modifications and improvements in analytical methods of cyanamide and its transformation products in the soil were attempted by the author. The ammonia nitrogen was determined by the aeration method using Mattew's aërator and a simple absorber (illustrated in original paper). In extracting the soil for the determinations of nitrate, urea, and dicyanodiamide nitrogen the addition of ferric sulphate or copper sulphate solution (ferric chloride solution in the case of urea determination) and then calcium hydroxide powder, to the soil sample, greatly facilitated the filtration and gave clear filtrate. The determination of nitrate nitrogen was made by reduction method, using, usually, Devarda's alloy, and, sometimes, zinc-copper couple or titanium trichloride solution, while the colorimetric method, using phenoldisulphonic acid, was occasionally employed. For the determination of urea nitrogen, the urease method was adopted, treating the soil extract or the soil itself with 5% water extract of Jack-bean flour. For determining cyanamide nitrogen in the soil the author devised a new method which comprises the conversion of cyanamide into urea by treating the soil sample with two times of 2 normal hydrochloric acid at 50∼55°C. for 3 hours. After nutralizing with sodium hydroxide the total urea in the filtrate was estimated by the urease method. The urea, which was present in the soil previous to the treatment with hydrochloric acid, having been determined separately, as described before, the cyanamide nitrogen was obtained by difference. As the sodium chloride, formed in the solution, has some depressing effect on the action of the urease, the urea decomposing power of Jack-bean flour extract in 5% solution of sodium chloride should be tested previously. This new method is also applicable to the analysis of commercial cyanamide, giving fairly satisfactory agreement with the volumetric silver cyanamide method and gravimetric dixanthyl urea method of Fosse. Lastly, the dicyanodiamide nitrogen in the soil, together with cyanamide nitrogen, was estimated by the method of Brioux, using the soil extract. The cyanamide nitrogen being determined separately by the author's method, the dicyanodiamide nitrogen will be obtained by difference.

The investigations were carried out with three soils of different types, viz, a fine sandy loam, a fine sandy loam rich in humus, and a fine clayey loam. Their mechanical and chemical analyses were thoroughly made, and some of their physical and chemical properties were also examined. The investigations on the changes of cyanamide and its transformation products in the soil were all conducted in bottle experiment, using half air dried fine soil passed through a 2 mm. sieve. The soils were weighed into 100 or 150 gms. lots of dry soil, allowance having been made for the moisture content of the soil. In order to approximate to the actual field practice, the use of higher doses of nitrogen compounds (which were to be tested) was avoided, but usually 5∼25 mgs. of N was given per 100 gms. of dry soil, with the exception of the experiment for dicyanodiamide formation from cyanamide, where application of 50∼100 mgs. cyanamide N was made. A series of lots receiving no nitrogenous compound served as control in most cases of experiments. Commercial cyanamide was usually sprinkled on to each lot of weighed soil placed on a sheet of paper, and the each was mixed and filled into wide-mouthed bottles. The other nitrogen compounds, viz. ammonium sulphate, urea, and dicyanodiamide, were always added, in aqueous solution, directly into bottles containing the weighed soil. The moisture content of soil was brought up to 1/2 and 1/4 of water capacity of the soil, in the "dry-field condition" and to the saturation point, that is, more than 100% of water capacity, in the "paddy-field condition". The bottles were then plugged with purified cotton-wool, and kept at temperatures varying 8∼35°C., each experimental temperature having been recorded continuously by selfrecording thermometer. The water lost by evaporation was replaced at intervals of 2∼14 days. A fresh bottle was used for each determination at the end of various periods.

Experimental results.

(1) Decomposition of cyanamide in the soil (with 3 tables in original paper.)

The three experiments, *effect of soil types*, *influence of temperature*, and *effect of method of applying the cyanamide*, were made, dry-field and paddy-field conditions being paralleled in all experiments. The effect of soil types and the influence of temperature upon the ammonia and urea formation from cyanamide were almost same in both soil conditions. The rate of ammonia production from cyanamide was somewhat slower in paddy-field condition than in dry-field condition ; and in the former condition the decomposition has stopped at ammonia formation stage, no nitrate having been formed. The effect of perfect and imperfect mixing of cyanamide with soil were distinctly different in both soil conditions, showing smaller effect in paddy-field condition than in dry-field condition. In the dry-field condition, the method of applying the cyanamide affected greatly the decomposition of cyanamide. Particularly, when the moisture content of soil was small and the dose of cyanamide was high the influence was marked, the ammonia formation from cyanamide being retarded considerably in the imperfectly mixed one. These results suffice to show the importance of soil water in bringing the cyanamide solution into contact with soil particles in order to transform the cyanamide, in arable soil. And in the actual dry-field practice, especially in the drought season, it is, therefore, desirable to distribute and work up the commercial cyanamide as uniformly as possible in the soil. Nevertheless, in the paddy-field practice the thorough mixing of cyanamide with soil might not be so important.

(2) Decomposition of urea in dry-field and paddy-field conditions (with 1 table in original paper).

Ammonification of urea in paddy-field condition was somewhat slower than in dry-field condition. But the conversion of urea into ammonia in both soil condition took place fairly rapidly; even at a relatively lower temperature (about 10°C.) 5 mgs. and 10 mgs. urea nitrogen per 100 mgs. dry soil were almost completely ammonified within 5～7 days and 7～14 days respectively. And after these periods the nitrification had begun in the dry-field condition, but in the paddy-field condition the decomposition had stopped at ammonia formation stage, as would be presumed.

(3) Dicyanodiamide formation from cyanamide in the soil (with 2 tables in original paper).

The two experiments, *effect of soil types* and *effect of doses of cyanamide*, were conducted. The effect of soil types upon dicyanodiamide formation from cyanamide was marked. With the fine sandy loam, the most inactive of the three soils used for the experiment the disappearance of cyanamide was slow, and more than 40% and 25% of added cyanamide remained unchanged at the end of 2 weeks and 5 weeks respectively, 50 mgs. cyanamide nitrogen per 100 gms. dry soil being added. And the increasing accumulation of dicyanodiamide was noticed, 27% of added nitrogen being transformed into dicyanodiamide at the end of 80 days. Whilst with the fine sandy loam rich in humus and with the fine clayey loam the disappearance of cyanamide was relatively rapid: the large parts of cyanamide disappeared within 2 weeks and 3 weeks respectively. And only small parts of cyanamide were transformed into dicyanodiamide, in both soils.

The dicyanodiamide formation from cyanamide depends largely on the quantity of cyanamide applied. Where the addition of 50 mgs. cyanamide nitrogen per 100 gms. dry soil was made the large parts of cyanamide were ammonified within 5 weeks at 20±5°C. in both soil conditions, and the formation of dicyanodiamide was very small, the fine clayey loam having been used as experimental soil. Whilst where the 100 mgs. cyanamide nitrogen was added to the same soil only three-fourth of added nitrogen was transformed into ammonia within the same period, and the remaining one-fourth was transformed into dicyanodiamide.

From above results, it is clear that the attempt of favourable conversion of cyanamide by mixing with ordinary soils previous to the application to the arable land would be vague and attend some dangers. For, as even with the active clayey loam, less than 2000 parts soil per 1 part cyanamide nitrogen, or about 400 parts soil per 1 part commercial cyanamide, dose not lead beneficially the decomposition of cyanamide without the formation of dicyanodiamide. But as a fertilizer for paddy-rice the case is different, for the dicyanodiamide exerts no harmful influence upon paddy-rice, as will be described later.

(4) The effect of dicyanodiamide upon the decomposition of cyanamide (with 2 tables in original paper).

Dicyanodiamide, when added at the rate of 0.5∼5.0 mgs. N per 100 gms. dry soil, matorially retarded or entirely stopped the nitrification, but did not interfered with the ammonia formation from cyanamide. These results agree with those of other authorities.

(5) Changes of dicyanodiamide in dry-field and paddy-field conditions, and the manurial value of dicyanodiamide for paddy-rice (with 3 tables and 1 figure in original paper).

These experiments were made with chemically pure dicyanodiamide prepared by the author from commercial cyanamide, showing 66.7% nitrogen by analysis and having good purity when determined as nickel guanyl urea.

The difference in rate of decompotion of dicyanodiamide between dry-field and paddy-field conditions was marked, the comparison being made by determining the produced ammonia and the remaining dicyanodiamide in the soil. In the dry-field condition the ammonification of dicyanodiamide took place slowly, and the influence of temperature was very slight. Whilst in the water-logged paddy-field soil condition the ammonia production from dicyanodiamide at relatively higher temperature (30±5°C.) proceeded fairly rapidly although at lower temperature (8∼16°C.) its ammonification and diminution took place much less rapidly.

These results show an intersting fact indicating that the exclusive fertilizing value of dicyanodiamide on paddy-rice may be due to its peculiar nature of decomposition in the soil. For in summer time——the season of rice culture and the prevailing temperature of paddy-field soil is then fairy high——the

Time elapsed in days.		Nitrogen present per 100 gms. dry soil (mgs.)							
		Dicyanodiamide added 25 mgs. N per 100 gms. dry soil.				Dicyanodiamide added 5 mgs. N per 100 gms. dry soil.			
		Dry-field condition		Paddy-field condition		Dry-field condition		Paddy-field condition	
		Ammonia N	$C_2N_4H_4$ N	Ammonia N	$C_2N_4H_4$ N	Ammonia N	$C_2H_4N_4$ N	Ammonia N	$C_2N_4H_4$ N
At low temperature (16—8—14°C.)	0	0.4	25.0	0.4	25.0	0.4	5.0	0.4	5.0
	30	0.8		0.8		0.6		0.6	
	75	1.2		1.2		1.0		1.1	
	120	1.2		1.5		1.0	4.4	1.6	4.4
	150	1.3	21.9	1.8	21.9	1.2	4.0	2.0	4.0
At high temperature (30±5°C.)	0	0.5	25.0	0.5	25.0	0.5	5.0	0.5	5.0
	25	1.4	24.3	2.0	23.0	1.4	3.3	1.4	2.6
	85	3.0	23.0	18.3	3.6	2.5	2.6	6.9	0
	135	4.0	15.1	22.3	0				

ammonification of dicyanodiamide in water-logged paddy-field soil seems to take place moderately to rapidly to meet the demand of rice plant.

It has long been an accepted fact that dicyanodiamide acts harmfully upon the plant growth when applied to the soil. On the other hand the good fertilizing value of dicyanodiamide on paddy-rice was reported by several experimenters in Japan (Aso, 1909; Tazaki and Taguchi, 1920). With the object of obtaining further information as to the fertilizing value of dicyanodiamide on paddy-rice the author carried out pot experiments with 1/200,000 hectare pots, using pure dicyanodiamide described before and ammonium sulphate for comparison. Each pot held 10 kilos equivalent dry soil of fresh fine sandy loam, allowance having been made for the moisture content of the soil. Dicyanodiamide and ammonium sulphate were tested at the three different rates of 1.0, 0.5, and 0.25 mg. N per pot respectively and applied on the day of transplantation of rice, a series of pots without nitrogen serving as control. A set of two pots was in each series and all received 10 gms. superphosphate and 4 gms. potassium sulphate. Three young plants of rice per pot were transplanted on July 9, and the crop was harvested on October 10, 1928. The yieldes (dry matter) of grain and strow of control (mean of two pots) were 11.8 gms. and 24.0 gms. per pot respectively, and nitrogen contained in them were 0.1205 gm. and 0.1674 gm. respectively. From these increased yields and availabilities of added nitrogen, etc. are calculated as follows.

Applied per pot		Increased yields of total crop (dry weights in gms.)	Relative increases of total yields	Increased yields of grain (dry weights in gms.)	Relative increases of grain	% added N removed in crop	Relative availabilities of added N
Ammonium Sulphate	1.0 gm. N	82 4	100	24.6	100	80.2	100
	0.5 gm. N	55.5	100	17.5	100	71.2	100
	0.25 gm. N	27.9	100	11.9	100	77.6	100
Dicyanodiamide	1.0 gm. N	66.4	80.5	31.9	137.8	65.8	82.0
	0.5 gm. N	59.2	106.6	27.4	156.5	74.0	103.9
	0.25 gm. N	33.9	121.5	17.4	146.2	95.6	123.1

The surpassing manurial value of dicyanodiamide over ammonium sulphate is generally observed in above results, and is more remarkable in the increased yields of grain. But, under the natural paddy-field condition the manurial value of dicyanodiamide would not be so high as in pot culture experiments. For dicyanodiamide is difficult to be retained in the soil, and well be easily washed out by drainage water through the soil.

The author considers that the exclusive fertilizing value of dicyanodiamide on paddy-rice, as against its harmful effect on most plants, is due briefly to the following facts :

(a) Paddy-rice has a somewhate greater resistance towards the harmful action of dicyanodiamide than dry-field (upland) crops.

(b) The indirect harmful action of dicyanodiamide, due to the retarding effect upon nitrification, does not affect the paddy-rice, as the nitrification does not occur essentially in the water-logged paddy-soil and is not needed on the paddy-rice culture.

(c) Under the soil condition of water-logged paddy-field in summer season, when the paddy-rice is grown, the dicyanodiamide decomposes so moderately that the formation of ammonia from it is well in accord with the growth of paddy-rice. Dicyanodiamide is made accessible by the paddy-rice only when it is transformed to ammonia. The direct assimilation of dicyanodiamide by the paddy-rice appears difficult, and necessitates further investigation.

Consequently, the secondary harmful after-effects of the application of commercial cyanamide, due to the dicyanodiamide which may be produced from cyanamide, are likely to be only slight or entirely absent in paddy-rice culture, although it is frequently experienced in dry-field crop culture.

ON THE OCCURRENCE OF A NEW AMINO ACID IN WATERMELON, CITRULLUS VULGARIS, SCHRAD.

By

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(Received April 10 th., 1930).

There are a number of investigations regarding the constituents of watermelon, but nitrogenous compounds of it are scarcely described. So the author studied them particularly and isolated a new amino acid and named it "Citrullin".

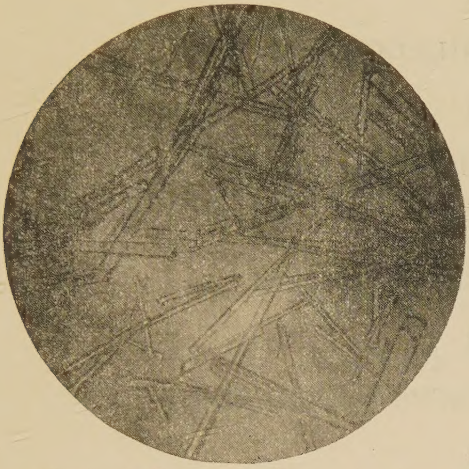
The analyses of the free compound as well as that of its copper salt have shown that the empirical formula should be $C_6H_{13}N_3O_3$.

The isolation of this compound was carried out as follows : ——

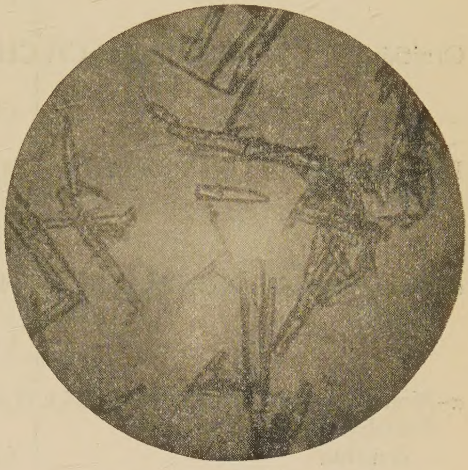
The pressed juice of watermelon was filtered and the clear colourless liquid was evaporated in vacuum. The resulting syrup was dissolved in water to 1 L. and a concentrated basic lead acetate solution was added to it as a preliminary precipitant. The nitrogenous constituents obtained from the filtrate with Neuberg's reagent (mercuric acetate, sodium carbonate, and alcohol) was again treated with phosphotungstic acid. The filtrate from the phosphotungstic acid precipitate, that is, the monoamino acid fraction contained "Citrullin". The copper salt obtained from this fraction was violet thin prisms, a little soluble in hot water and decomposed at 257~8°. The yield of it was 3.4 g. from 2 kg. syrup. The free amino acid obtained from the copper salt is colourless thin prisms decomposing at 202°, but 205~6° in the sealed capillary. It is easily soluble in water but not at all in pure ethyl and methyl alcohols, ether, and the other organic solvents. The aqueous solution is nearly neutral to litmus, and possessed a slightly sweet taste. Showing no rotatory power, this compound seems to be racemic. It is precipitated in a concentrated solution with phosphotungstic acid, but the precipitate is easily soluble in the excess of the reagent. It is also precipitated with mercuric salts such as acetate, nitrate, sulphate, and chloride in an alkaline solution ; but not with phosphomolybdic acid, tannin, and basic lead acetate.

The free compound gives reactions with ninhydrin, Ehrlich's and Schiff's reagents and with phenol and sodium-hypochlorite ; but it does not give following reactions ; Biuret, Diazo, Millon's, Jaffe's, Sakaguchi's, Folin's, Adamkiewicz's, and Mörner's reactions. This compound is not hydrolysed with sulphuric or hydrochloric acid, and has no properties of an anhydride or a peptide.

Two third of its nitrogen is liberated by the Van Slyke's method, and one third is titrated by the Sörensen's formol method. And so this has two amino and one carboxyl groups.



Citrullin (δ–Carbamyl ornithin). 1 : 165.



Copper salt of Citrullin. 1 : 770.

The author, taking into consideration the fact that there exist pairs of amino acids from the difference of one oxygen molecule, alanine and serine, phenylalanine and tyrosin, proline and oxyproline, glutamic acid and oxyglutamic acid and lysine and oxylysine, first suspected that the new amimo acid might be a derivative of arginin, of which one amino group was substituted by an oxy group. On preparing arginic acid, and comparing its properties with the above compound, a big difference was noticed, especially, in the fact that the former is decomposed by acids and the latter by weak alkalies. The new compound is not, therefore, surely arginic acid. From the following reasons, the author proposes the formula.

$$NH_2\text{-}CO\text{-}NHCH_2CH_2CH_2CH(NH_2)COOH$$

to the compound now in consideration.

(1) α–Amino δ–oxyvalerianic acid gives a copper salt, but δ–amino α–oxyvalerianic acid does not.

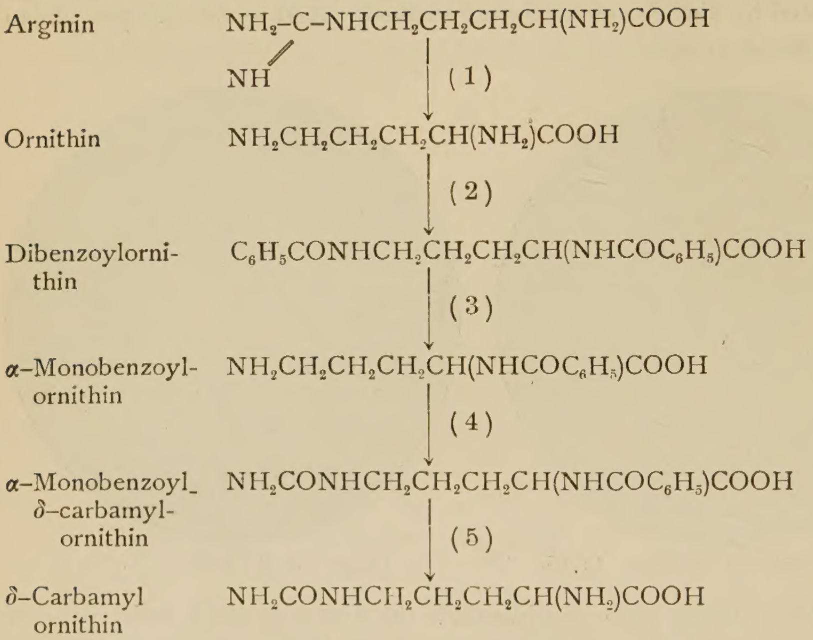
(2) Arginin gives a copper salt, but arginic acid does not. The new amino acid must have, in all probability, an α–amino group.

(3) The carbamyl group attached to α–amino group forms an anhydride by mineral acids, but the new amino acid does not.

(4) By the action of alkalies, the new amino acid gives off ammonia and is converted into ornithin.

(5) It gives reactions of urea such as Ehrlich's and Schiff's reactions. Hereupon, the author synthesized the compound of the above constitution by

the following five steps.



Arginin $NH_2-\underset{NH}{C}-NHCH_2CH_2CH_2CH(NH_2)COOH$

$\downarrow$ (1)

Ornithin $NH_2CH_2CH_2CH_2CH(NH_2)COOH$

$\downarrow$ (2)

Dibenzoylornithin $C_6H_5CONHCH_2CH_2CH_2CH(NHCOC_6H_5)COOH$

$\downarrow$ (3)

α–Monobenzoyl-ornithin $NH_2CH_2CH_2CH_2CH(NHCOC_6H_5)COOH$

$\downarrow$ (4)

α–Monobenzoyl_ δ–carbamyl-ornithin $NH_2CONHCH_2CH_2CH_2CH(NHCOC_6H_5)COOH$

$\downarrow$ (5)

δ–Carbamyl ornithin $NH_2CONHCH_2CH_2CH_2CH(NH_2)COOH$

The synthesised product showed the same crystal form and the same decomposition point with the natural one, and properties and reactions of the both were same in every respect.

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